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Nevsky, L. S., "On Atypical Forms of B. Anthracis",
Agrobiologiya, #1 (79), Moscow, 1953,
pp. 18-22.
A. Pirsconok, translator.

Variability in bacterial forms was noted by Russian scientists as early as the 30's of the last century. Tsenkovsky's work on anthrax vaccine I and II was the classical example of the directed variability of microbes.

The role of Russian scientists is especially significant in the study of the biology of the agent of anthrax. So, as early as 1888, N. P. Ganaloye, and somewhat later, P. N. Andreev, S. N. Vyscholosky, V. M. Kirsanov, N. A. Nikulin, and in recent years, F. A. Terentev, D. M. Teternik and others, showed convincingly, that the anthrax bacilli tolerate profound variations under the influence of certain factors. The direct influence of media on the formation of variants of B. anthracis and other kinds of microbes was demonstrated both on artificial nutritive media as well as in the live organism. Variability of microbes may be produced by various internal factors, physical and chemical influences, in passage through live organism, by the influence of other kinds of microorganisms or their products of secretion and by the influence of bacteriophage. In the practice of diagnostic laboratories significant deviations from typical anthrax bacilli are also observed frequently. In the investigation of pathological materials from pigs and other animals, at times we isolated non-characteristic strains of anthrax bacilli with respect to morphological, cultural and biological properties.

In the microscopic study of preparations taken directly from animal organs as well as from derived cultures, we observed forms which were swollen, distorted, very thick or very thin, non-capsulated or consisting only of single capsules of anthrax bacilli. We frequently

discovered a bacillary involution, remnants of them, and a weak stainability. At times the quantity of bacilli was insignificant and in certain cases in the preparations there were absolutely no impressions of them directly from the material.

In growing cultures on meat-peptone agar we often detected slightly spiral colonies which in form were completely different from typical anthrax. Certain strains produced hemolysis on blood media, others made the meat-peptone broth cloudy. In separate cases, in the presence of capsulated forms of *B. anthracis* in initial smears the number of colonies in the plantings was so insignificant that 20 bacteriological plates were required to obtain a growth of a single colony of anthrax bacilli on 2-3 of them.

We will describe the atypical strains of anthrax bacilli that we obtained:

1. Strain No. 3125 had a clearly marked motility. It was isolated from pigs with the following clinical picture: anorexia, temperature - 41-5°, a hot, hard swelling in the neck region (on the right side). Pathological examination (post-mortem) of the animal showed an edema of the throat and neck, hyperemia of the lymph nodes and a bad exsanguination of the carcass. Microscopic study of preparations from materials of the lungs and lymph nodes displayed bacilli (some with capsules), but preparations from the lymph nodes of the neck showed a large quantity of bacilli strung out in strings, and twisted and interlaced.

Growth on meat-peptone agar in the first 24 hour period was typical; in the second 24 hours they started to become somewhat curled and the colonies became rounder. The growth on broth was typical, like a piece of cotton in clear media. Hemolysis was not observed on blood media. On gelatine, the growth was "herringboned", on a potato--covered with

gray. Milk curdled in 4-6 days. Litmus became slightly red and then colorless. Precipitation reaction with an antigen obtained from strain No. 3125 and precipitated with serum was positive in a dilution to 1,10,000.

Biological checks of strain No. 3125 on 38 white mice, 3 guinea pigs and 3 rabbits showed it to be a pathogen: all infected animals succumbed to a septic form of anthrax. White mice died (on an average in 2-3 days) even from doses of 0.0001 ml of a 24 hour broth culture of this strain.

The single difference between strain No. 3125 and the typical anthrax cases was the active motility of the bacilli. In studying suspension droplets from cultures of this strain for motility, there was noted a clear forward motion of the bacilli.

We were not able to obtain cultures from one microbe cell and study it for motility. In order to bridge this gap, we planted a fractional culture on agar plates and studied single isolated colonies. With passage on different media, infection of experimental animals and then the later study of the cultures in suspension droplets, invariably an active motility was observed. Periodically, strain No. 3125 was planted on meat-peptone broth and in the course of 2 years, it retained its motility.

This all says that in nature the existence of motile strains of anthrax bacilli is possible.

The results of our studies do not differ from the data obtained by both Soviet and foreign researchers (Teternik, Williamovsky, Prozhevsky, Klimova, Chonots, Yanushke etc.).

2. Avirulent Strain No. 45 was isolated from the submandibular lymph nodes of a pig which had no clinical evidence of illness. But with a veterinary-sanitary consultation on the carcass, evidences of a

pathological process were noted in the left submaxillary lymph nodes, typical for a local form of anthrax; the consistency of the lymph nodes was compact; on cross-section, their color was brick-red with necrotic sections.

Microscopic study of preparations from the affected lymph nodes showed a small amount of thick, short, swollen little chains and long strings, some taking stain poorly. In plantings on meat-peptone agar, there was noted a growth of dull gray, curled colonies, characteristic for anthrax. In the study of suspension droplets, motility was not observed. Planted on meat-peptone broth, the growth was typical, like a piece of cotton in clear media. Hemolysis was observed on blood media only in 7-10 days. On gelatine, the growth was "herringboned" and on a potato, there was a gray cover. Milk curdled in 4-6 days. Litmus turned colorless on the 3rd day. The local form of anthrax (submaxillary lymph nodes) was supported bacteriologically. Cultures of materials from vital organs and other lymph nodes did not yield *B. anthracis*.

Precipitation reaction with an antigen prepared from an agar culture of strain No. 45 and precipitated with serum was positive in a dilution up to 1:10,000. Pathogenic properties of the given strain were studied on 26 white mice, 3 guinea pigs, and 3 rabbits. All the experimental animals lived. The white mice were infected with a subcutaneous or intraabdominal dose from 0.1 to 1.0 ml of a 24 hour broth culture; rabbits and guinea pigs--with a dose up to 3 ml.

Under the directorship of Professor F. A. Terentyev, we studied the immunogenic properties of strain No. 45 on white mice and rabbits. A vaccine was prepared from this strain in two variations: (1) a suspension of the spores in normal saline and (2) the same suspension as (1), but with the addition of aluminum hydroxide in the relationship

1:2 (i.e. one part aluminum hydroxide to two parts of spores of the avirulent culture in normal saline). The aluminum hydroxide was supposed to serve as a stimulating substance. The results of the experiment appear in Table 1.

Table 1
Vaccination of White Mice With the Avirulent
Culture of Strain No. 45. The vaccine was given
subcutaneously in 0.2 ml doses.

Vaccine	No. of times Vaccinated		No. of White Mice	Controlled Infection*	
	1 time	2 times		Date	Date
	Date				
Variant # 1	April 20	May 7	6	May 15	3 dead 3 alive
Variant # 2	April 30	May 7	5	May 15	1 dead 4 alive
Variant # 1	May 7	-	5	May 15	all dead
Variant # 2	May 7	-	5	May 15	1 dead 4 alive
Control	-	-	5	May 15	all dead

* Infection was done with 1 vaccine of Sonovsky in a dilution of 1:10, dose 0.2 ml.

As is evident in Table 1, with two vaccinations of one avirulent culture, there was immunity in half of the white mice; by adding aluminum hydroxide to the culture, 4 out of 5 developed immunity; and with a single vaccination there was an immunity only in the group in which there was a shock made with the vaccine with a stimulating substance (4 out of 5 mice survived).

The results of the experiment definitely indicate that two vaccinations with the avirulent culture (and in cases where a stimulating substance was added--with a single vaccination) transmit a stable immunity to white mice.

It is necessary to note that, up to now there has been no vaccine preparation from a live culture of *B. anthracis* which would protect mice from anthrax infection, and in this respect, our experiments are the first. The immunogenic properties of strain No. 45 were also checked on rabbits (Table 2). (page 7).

From Table 2 it follows that the avirulent culture transmits an immunity to rabbits, too; the immunity is more stable in the variant to which aluminum hydroxide has been added than in the variant without a stimulating substance.

The data of our orientation experiments show on the one hand, that strain No. 45 belongs to anthrax strains, but on the other hand, that the avirulent culture of *B. anthracis* Strain No. 45 may be used as a vaccinating strain. For this reason, it is necessary to study it further.

3. Strain No. 1517 was isolated from a slaughtered pig.

Microscopic studies of cultures taken directly from the organs of this pig displayed single rods (bacilli) with a capsule, their involution and traces of them. In cultures on meat-peptone agar in only 3 dishes out of 20 there was noted a growth of several wavy-edged colonies which were faintly spiral-like. Only after being planted on other media--meat-peptone broth, meat-peptone gelatine, blood media, potatoes, litmus--a growth was observed which was characteristic for *B. anthracis*. Two white mice infected with the culture died only after 120-144 hours.

Table 2

Vaccination of Rabbits with an Avirulent Culture of
 B. Anthracis Strain No. 45. The vaccine was administered
 subcutaneously in 1 ml doses.

Group	Vaccine	No. of Times Vaccinated		No. of the Rabbit	Controlled Infection*	
		Once	Twice		Date	Result
1	Variant (1)	April 30	May 7	7031	May 20	Dead in 48 hours
		April 30	May 7	7034	May 20	Alive
2	Variant (2)	April 30	May 7	7032	May 20	Alive
3	Control	-	-	2 rabbits	May 20	Dead in 48 hours and in 72 hours

* Infection was produced with a standard virus CMI, series No. 6 in a dilution
 of 1:100, and an 0.5 ml dose.

Typical anthrax colonies were obtained from the dead mice. Mice infected with these died in 36-48 hours.

4. Strain No. 3601 was isolated from a pig which was killed because of suspected erysipelas. Microscopically, smears from the liver, kidneys and lymph nodes showed a significant number of short little chains consisting of exceptionally thin non-capsulated bacilli. But the growths on meat-peptone agar, meat-peptone broth and meat-peptone gelatine, blood media, and litmus were typical of *B. anthracis*. On the potato a yellowish-covered growth was noted. White mice infected with the culture died in 96-120 hours.

5. Strain No. 6351 was isolated from a slaughtered cow. Two-three short, non-capsulated bacilli were isolated from preparations from the lymph nodes. Cultures on meat-peptone agar showed a growth of individual opaque colonies, with a slight tendency to curl and a center darker than the periphery. On meat-peptone broth, meat-peptone gelatine, a potato and in litmus, the growth was typical for *B. anthracis*. On blood medias, hemolysis took place in 3 days. White mice infected with the culture succumbed in 20-40 hours. The precipitation reaction was positive. A characteristic property of the strain is hemolysis.

6. Strain No. 5661 was isolated from the muscles of an ox. In preparations from the muscles irregularly stained little chains were observed. Cultures on meat-peptone agar had atypical colonies with a dark center and some twisting. In the first 24 hour period, on meat-peptone broth there was "a piece of cotton" and in the second 24 hour period--turbidity. On meat-peptone gelatine and blood media, in litmus and on a potato, the growth was typical for *B. anthracis*. One of two white mice infected with the culture died in 144 hours, the other survived. The precipitation reaction was positive. In the original

cultures from the muscles, growths of intestinal bacilli and *proteus vulgus* were observed along with the *B. anthracis*.

All our materials point to the fact that not all cases of anthrax may be attributed to one of the typical cases described in the handbooks. From here it is clear that the accepted classification of bacteria based on the principle of monomorphism is inadequate.

In the variability of microbes, the role of various conditions of external media and the role of the condition of the animal is unquestioned. For this reason, the complex method of research must be placed at the foundation of laboratory diagnostics: to define all of the properties of the microbe, to take into consideration the clinico-epizootological and pathological-anatomical data, and with this, certainly to take into account that an atypical form of the agent may also be identified.

We consider that the existing ideas on the so-called pseudo-anthrax microbes (anthracoid or anthrax-like) need to be re-examined, especially for such strains of the anthracoids which are isolated from materials accompanying a pathological-anatomical picture characteristic of anthrax.

It is necessary to correct descriptions of symptoms and properties of similar kinds (anthrax and pseudo-anthrax microbes) contained in handbooks.

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